Mode of Action Studies Do Not Support Distant Site Carcinogenic Targets for Formaldehyde

Comments by:

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Who am I? I am Dr. Melvin Andersen, Director of the Program in Chemical Safety Sciences at The Hamner Institutes for Health Sciences, Research Triangle Park, NC. I have worked in toxicology and risk assessment with various organizations since receiving my PhD in Biochemistry and Molecular Biology from Cornell University, Ithaca, NY and joining the US Navy Toxicology Unit, Bethesda, MD in July 1971. My current organization was previously known as CIIT, the Chemical Industry Institute of Toxicology. Over the past 30 years, scientists at CIIT and The Hamner have conducted a broad variety of toxicity testing and toxicity research to unravel the mode of action by which inhaled formaldehyde causes toxic and carcinogenic responses in the rat nose. CIIT/Hamner staff published the first two-year bioassay with formaldehyde (Kerns et al., 1983), conducted a repeat cancer study with evaluations of multiple endpoints at several sampling times during the two-year exposure (Monticello et al.,1991) , established detailed protocols for more fully evaluating nasal responses of inhaled gases (Mery et al., 1994), developed airflow models to account for local uptake of formaldehyde in the nose (Kimbell et al., 1993), developed models describing the formation of DNA-protein cross-links within the target tissues (Casanova et al.,1994), and applied two-stage clonal growth models for cancer risk assessment to account for both toxicity (cell proliferation) and DNA-reactivity in the carcinogenic process with formaldehyde in the nose (Conolly et al., 2003)

In the past several years, Hamner scientists, including myself, have been involved in assessing the time course and dose dependencies of genomic changes induced in target tissue areas of the nose caused by inhalation of formaldehyde for periods up to 90-days. These studies required new tools for assessing functional changes in groups of genes, including the development of genomic benchmark dose modeling procedures (Yang et al., 2009). Our studies evaluated regions of exposures below those causing toxicity, as well as concentrations used in the original inhalation studies. Results from our one-day and from our three week exposure studies have been published and both papers were cited as papers of the year by the Risk Assessment Specialty Section of the US Society of Toxicology (Thomas et al., 2007; Andersen et al., 2008). My comments focus on the implications of this fuller body of mechanistic work for assisting decisions about classification of the carcinogenic potential of formaldehyde in humans.

What is Formaldehyde? Formaldehyde is not simply an exogenous, highly irritant gas. It is a normal product of intermediary metabolism in mammals, formed endogenously from serine, methionine, choline, and glycine by demethylation of N-, O-, and S-methyl compounds and is found in blood and tissues at concentrations between 0.1–0.2mM (Heck et al., 1982, 1985). While formaldehyde can interact reversibly with various macromolecules, it readily and reversibly reacts with glutathione to form hydroxymethylglutathione which is the substrate for formaldehyde dehydrogenase, the enzyme that converts formaldehyde to formic acid.

Glutathione is not consumed in this reaction. In most tissues glutathione concentrations exceed 1 millimolar. The normal state for all cells is a background of formaldehyde whose intrinsic reactivity is counterbalanced by chemical processes that restrict increases in free formaldehyde concentrations in cells. Importantly for subsequent discussions, these background levels of formaldehyde persist in all cells in the body.

Nasal Responses to Formaldehyde: At sufficiently high concentrations, airborne formaldehyde is a potent eye and respiratory tract irritant. The mouse RD50, that is, the inhaled concentration causing a 50% decrease in respiratory rate, was 3.1 ppm (Buckley et al., 1984). The inhalation concentrations eliciting carcinogenic responses in rats were above the RD50, i.e., they were at concentrations that were highly irritating to the epithelial surfaces of the nose. Due to its high reactivity with water, formaldehyde is readily taken up into the mucosal surfaces of epithelial tissues as it passes through the nose with a significant anterior to posterior concentration gradient along the nasal epithelium (Kimbell et al., 1993). Epithelial surfaces predicted to have the highest uptake correspond to those areas that are most affected by formaldehyde toxicity and carcinogenicity. In 2-year inhalation studies, formaldehyde increased squamous cell cancer in the proximal regions of the nose of rats (Kerns et al., 1983; Monticello et al., 1996) at concentrations of 6 ppm or higher. At 6 ppm, cancer incidence was low. Only 1 tumor was observed across the two bioassay studies. The incidence rose sharply between 6 and 15 ppm; half of the exposed rats at 15 ppm developed nasal tumors.

Dose-Dependent Transitions expected with formaldehyde in all cells: Formaldehyde responses of tissues are expected to show dose-dependent transitions (Slikker et al., 2004). Since nasal tissues already have a significant level of endogenous formaldehyde, low concentration exogenous exposures will not cause appreciable increase in intracellular or intranuclear formaldehyde above endogenous levels. At some intermediate inhaled concentration (in the vicinity of 2 ppm), the exposure may lead to local increases in tissue levels at the front of the nose and initiate the first signs of cellular responses to formaldehyde. At sufficiently high inhaled concentrations, i.e., 6 ppm and above, intracellular concentrations at sites of high uptake in the nose or upper respiratory tract increase significantly and produce cytotoxicity and inflammatory responses, thereby enhancing cell proliferation, cross-linking and carcinogenicity as noted in the long-term repeated exposures. Key questions for risk assessment and for classification of formaldehyde are to ascertain the concentrations and exposure durations where perturbations caused by formaldehyde are sufficient to lead to initial biological responses and where they become large enough to cause frank cytotoxicity, excessive cross-linking, and carcinogenicity. Another requirement in assessing the consequences of dose-dependent transitions for classification and risk assessment is to ascertain the qualitative relationship between the initial cellular responses compared with the

cytotoxic, proliferative tissue responses to high concentration formaldehyde. Are similar patterns of response seen at all concentrations with diminished incidence at lower concentrations or are patterns of response qualitatively distinct between low and high exposures? Our research strongly supports the latter conclusion with attendant implications for risk assessment and classification for the nose and for other tissues in the body.

Dose-Dependent Transitions for Tissue Targets: In the past, dose-response studies with formaldehyde primarily evaluated histopathology, DNA-protein adducts, and cell proliferation. The emergence of genomic profiling provides other tools to examine tissue responses at levels below those causing overt toxicity and to further evaluate dose-dependent changes in tissue responses to formaldehyde. In our genomic studies (Thomas et al., 2007; Andersen et al., 2008; Yang et al., 2008) tissue pathology served as a phenotypic anchor for interpretation of the microarray results. We assessed the time course of tissue changes over a 3-week exposure period with inhalation exposures similar to the three lowest concentrations used in the cancer bioassays— 0, 0.7, 2, and 6 ppm, 5 days/week for up to 3 weeks. In addition, a more complete dose-response study was accomplished for single 6-h exposures by adding groups for 15 ppm inhalation and for 40uL, 400 mM instillation. Our experimental design allowed for (1) observation of time dependence of histopathology and gene changes for a 3-week period over a concentration range from 0.7 to 6 ppm; (2) evaluation of the dose response following single inhalation exposures up to 15 ppm; and (3) comparison of instillation with high concentration inhalation or instillation.

Evaluation in the region of concentration below those that are carcinogenic in a large proportion of exposed animals could possibly extend the dose response curve for precursor responses to lower concentrations. However, in our three week study design, the genomic changes did not prove to be more sensitive than tissue responses. (A 90-day genomic study, now completed but not yet completely analyzed, reaches similar conclusions). No significant gene changes were observed at 0.7 ppm and very few were observed at 2 ppm. The 2 ppm changes seen at 5 days were resolved by three weeks. Transient squamous metaplasia was noted suggesting tissue adaptation and reduced tissue sensitivity by day 15 for the rats in the 2 ppm exposures. Interestingly, the most sensitive targets (affected at the lowest concentrations) appear to be associated with the external cell membrane and cell-matrix interactions. These mechanistic studies on gene expression during short-term repeated exposures to formaldehyde indicated that the most sensitive responses to formaldehyde are likely to be associated with reactivity of formaldehyde with cell components on the plasma membrane or in the extracellular matrices surrounding the epithelial cells (Andersen et al., 2008). Differential responses of the cell exterior and cell interior are not unexpected due to lower concentrations of protective molecules, such as glutathione and lower levels of constitutive enzymes, such as

formaldehyde dehydrogenase, outside the cell. Qualitatively, the targets of formaldehyde appear to change with increasing exposure concentrations and consequences of reactivity with nuclear proteins become evident only at the highest exposure concentrations.

Contrasting High Dose Instillation and Inhalation: Hester et al. (2002, 2003) assessed gene expression in rat nasal epithelium after instillation of extremely high local doses of a formaldehyde solution (40 ul of 400 mM) using a custom array with a restricted number of genes. No dose response information was developed. At these high tissue doses, gene families related to DNA damage and repair were upregulated. In our comparisons, instillation affected a much larger suite of genes and gene families than any of the inhalation concentrations, even 15 ppm. These instillation results do not appear to be directly relevant for decisions about respiratory tract risks at low inhaled concentrations or for risks in diverse tissues in the body that receive little of no extra tissue formaldehyde from an inhalation exposure.

Extrapolations beyond the Site of Contact: Formaldehyde, a normal metabolite in all cells, has dose-dependent transitions due to endogenous pathways that counter its reactivity. Patterns of delivery, i.e., with uptake first enhancing extracellular formaldehyde, lead to preferential responses of extracellular tissue components. Intracellular targets only appeared to become affected at higher concentrations consistent with cytotoxicity of formaldehyde. At concentrations below overtly cytotoxic concentrations, excursions in cellular formaldehyde will not increase intracellular formaldehyde. Without these excursions in tissue cellular formaldehyde, cell constituents in the epithelium do not show cytotoxicity or DNA-mutations. This conclusion would be equally valid for other cell types within the nasal epithelium, including hematopoetic cells with their own endogenous background levels of formaldehyde.

Despite the highly irritating nature of formaldehyde, the original bioassays had exposures as high as 50 ppm. If a protocol was brought to an IACUC (Intuitional Animal Care and Use Committee) in 2009 to conduct inhalation exposures at concentrations of a substance generating intense respiratory tract irritation, such a study would be unlikely to be approved. Highly irritating exposure concentrations would be regarded to be in excess of a Maximum Tolerated Dose. At the very least, approval would require very detailed justification. Formaldehyde at concentrations without extreme irritation (6 ppm and below) would not even be classified as a rodent carcinogen. Results over the past thirty years remain consistent – formaldehyde at concentrations below those causing intense mucosal irritation is not expected to be a respiratory tract carcinogen, even in experimental animals. Similarly, at these concentrations, formaldehyde would not be expected to cause remote site effects or be able to cause effects by mutation of migration-capable cells in the epithelium that could go on to seed remote neoplasia.

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